2005. The Journal of Arachnology 33:569–572

ON THE OCCURRENCE OF THE 9 + 0 AXONEMAL PATTERN IN THE SPERMATOZOA OF SHEETWEB SPIDERS (ARANEAE, LINYPHIIDAE)

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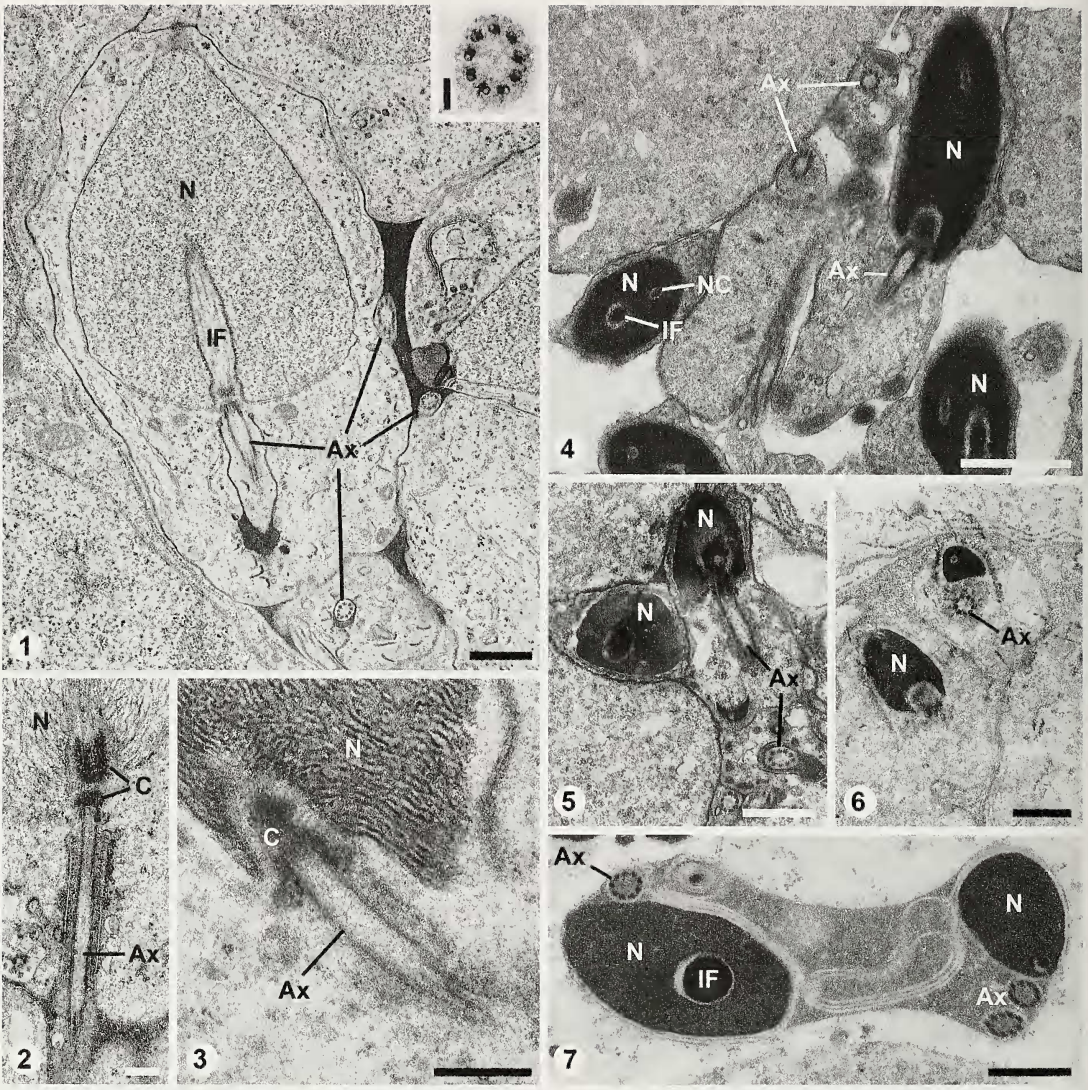
ABSTRACT. In general, flagella and cilia of eukaryotes show an axoneme composed of a 9 + 2 microtubular pattern. However, the axoneme of spider spermatozoa is characterized by an exceptional 9 + 3 microtubular pattern, which is known as a synapomorphy of the Megoperculata (Amblypygi, Uropygi and Araneae). In contrast to all other observed spiders, the axoneme of the linyphiid spider *Linyphia triangularis*, was shown to lack the central microtubules thus representing a 9 + 0 axoneme. In the present study, we investigated the spermatozoa from several linyphiid species of different genera in order to show whether this peculiar pattern also occurs in other linyphiid spiders. Interestingly, in all observed species (*Neriene clathrata*, *N. peltata*, *Linyphia hortensis*, *Lepthyphantes* sp., *Oedothorax gibbosus*, *Gongylidium rufipes* and *Drapetisca socialis*) we found the 9 + 0 microtubular pattern in the axoneme. Since this study, although considering still a very limited number of species, includes species from Linyphiinae (Linyphiini and Micronetini) and Erigoninae it seems likely that this pattern is an autapomorphy of Linyphiidae.

Keywords: Sperm, phylogeny, axoneme, microtubules

The typical and plesiomorphic axoneme found in eukaryote flagella and cilia possess a 9 + 2 arrangement of the microtubules. Nevertheless, there is a wide range of modifications within the axoneme of sperm flagella, e.g., in insects (Jamieson et al. 1999). Also within the Chelicerata a broader range of patterns occurs as shown by the recent species of the early derivative group, Xiphosura, where two different patterns are reported (9 + 2 and 9 + 0; Fahrenbach 1973; Yamamichi & Sekiguchi 1982; Alberti & Janssen 1986). Within arachnids only the spermatozoa of Scorpiones, Uropygi, Amblypygi, Araneae, Pseudoscorpiones and Ricinulei possess a flagellum (summary in Alberti 2000), in contrast to the spermatozoa of Solifugae, Acari, Palpigradi and Opiliones, which are aflagellate (the only exception is the opilionid genus *Siro* which shows an axoneme during the spermatogenesis; Juberthie et al. 1976; Alberti in press). The typical 9 + 2 pattern occurs only in Scorpiones, Pseudoscorpiones and Ricinulei. However, in Scorpiones aberrant patterns, e.g., 9 + 0 and 9 + 1 have also been reported (Hood et al. 1972; Jespersen & Hartwick 1973; Alberti 1983). The Uropygi, Amblypygi and Araneae (Megoperculata) possess as a synapomorphy a 9 + 3 pattern (summary in Alberti 2000; Michalik et al. 2003, 2004, in press and further personal observations). Thus it seems remarkable, that the linyphiid spider *Linyphia triangularis* (Clerck, 1757) has an unusual 9 + 0 axonemal pattern (Alberti 1990); unfortunately until now there have been no other ultrastructural observations on Linyphiidae spermatozoa to know assess if this pattern is typical of this taxon.

In the present study, we investigated the spermatozoa of several different linyphiid spiders from the subfamilies Linyphiinae (Linyphiini and Micronetini) and Erigoninae to begin a determination of the generality of this peculiar axonemal pattern within the Linyphiidae.

Male specimens of *Neriene clathrata* (Sundevall 1830), *N. peltata* (Wider 1834), *Linyphia hortensis* Sundevall 1830 (Linyphiinae, Linyphiini); *Lepthyphantes* sp. (Linyphiinae, Micronetini); *Oedothorax gibbosus* (Blackwall 1841) and *Gongylidium rufipes* (Linnaeus 1758) (Erigoninae); and *Drapetisca socialis* (Sundevall 1833) were dissected and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buff-



Figures 1–7.—Spermatozoa of the observed linyphiid species. 1. Early spermatids of *Oedothorax gibbosus* with axonemes in cross—and longitudinal sections. Scale bar = 1 μm. Inset: Detail of the axoneme of *Linyphia hortensis* in cross-section showing the 9 + 0 pattern. Scale bar = 0.1 μm. 2—3. Posterior part of an early spermatid in longitudinal section. 2. *Neriene clathrata*. Scale bar = 0.25 μm. 3. *Lepthyphantes* sp. Scale bar = 0.5 μm. 4—6. Late spermatids. 4. *Drapetisca socialis*. Scale bar = 1 μm. 5. *Gongylidium rufipes*. Scale bar = 0.5 μm. 6. *Neriene peltata*. Scale bar = 0.5 μm. 7. Coiled spermatid of *Linyphia hortensis*. Scale bar = 0.5 μm. Abbreviations: Ax = axoneme, C = centriole, IF = implantation fossa, N = nucleus, NC = nuclear canal (canal containing the acrosomal filament).

er followed by postfixation in buffered 2% osmium tetroxide. After rinsing, the specimens were dehydrated in graded ethanols and embedded in Araldite or Spurr's resin (Spurr 1969). Ultrathin sections were made on a Leica ultramicrotome and the sections were stained with uranyl acetate and lead citrate (Reynolds 1963). The examination was performed with a Zeiss EM 10A electron microscope. Voucher specimens have been deposited in the Zoological Museum of the University of Greifswald.

Spermiogenesis starts with spermatids which are mainly characterized by a large, roundish nucleus lying in a homogenous cytoplasm (Fig. 1). The axoneme migrates into the posterior pole of the nucleus (Figs. 1–4). The centrioles are orientated in the tandem po-

sition (Fig. 2). In all sections, the absence of the central tubules in the axoneme is obvious in all investigated species (Figs. 1–7) and in cross sections, the 9 + 0 axonemal pattern is evident (Figs. 1 inset, 4–7). Parallel to the migration of the axoneme, a deep posterior indentation into the nucleus is formed, the so-called implantation fossa (Fig. 1) which is filled with dense material at the end of spermatogenesis (Figs. 4, 7). At the end of spermatogenesis the nucleus coils once and the axoneme turns around the nucleus in the periphery of the cell (Fig. 7).

The occurrence of the 9 + 0 axonemal pattern is unique among the spermatozoa of Megoperculata and was first shown by Alberti (1990) for *Linyphia triangularis*. In spiders a 9 + 3 pattern normally occurs and was studied in detail by Dallai et al. (1995). The present study shows the peculiar 9 + 0 pattern for all observed Linyphiidae. Based on these observations many questions arise concerning the function and the phylogenetic impact of this character. Unfortunately, no studies on the movement of linyphiid spermatozoa exist. However, it was shown from other animal species that an axoneme which lacks the central tubules can still move. For example, Ishijima et al. (1988) compared the beat pattern from Asian and American horseshoe crabs. The Asian species *Tachypleus gigas* (Müller 1785) possess a 9 + 0 axoneme which beats in helical waves, in contrast to the planar waves of the 9 + 2 axoneme of the American horseshoe crab *Limulus polyphemus* (Linnaeus 1758). Similar results were also reported in the detailed studies of Gibbons et al. (1983, 1985) on the eel *Anguilla anguilla* (Linnaeus 1758) which possess spermatozoa with a 9 + 0 axoneme which lacks the central tubules as well as other structures, e.g., outer dynein arms, radial spokes and spokeheads. The spermatozoa of the eel beats in helicoidal waves. Hence it can be assumed that the spermatozoa of Linyphiidae are motile and the movements of the axoneme are different from those of other spider spermatozoa that possess a 9 + 3 axoneme. More investigations are needed to test this hypothesis and clarify the possible influence (selective advantage?) within the female genital system.

Furthermore, the occurrence of the 9 + 0 axonemal pattern in all observed species of linyphiids supports the assumption of Alberti (1990) that this peculiar pattern might be an autapomorphy of Linyphiidae. Therefore it would be of much interest to know the situation in the supposed sister taxon Pimoidae as well as in the other linyphiid subfamilies (e.g., Hormiga 1994a, b; 2000).

ACKNOWLEDGMENTS

We are very grateful for the assistance of Wencke Reiher. Furthermore, we thank Gabriele Uhl (Bonn) for the provision of the males of *O. gibbosus*. For the exact identification of part of the material we are indebted to Konrad Thaler (Innsbruck).This study was financially supported by the German National Merit Foundation (grant to PM) and the German Research Foundation (DFG, Al 138/11–1).

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Manuscript received 27 September 2004, revised 13 August 2005.